# Gene Regulation Networks for Modeling *Drosophila* Development

## Draft Chapter for Bolouri/Bower Book

Eric Mjolsness
Machine Learning Systems Group
Caltech Jet Propulsion Laboratory
Pasadena CA 91109

## 1 Introduction

This chapter will very briefly introduce and review some computational experiments in using trainable **gene regulation** network models to simulate and understand selected episodes in the development of the fruit fly, *Drosophila melanogaster.* For details the reader is referred to the papers introduced below. It will then introduce a new gene regulation **network model** which can describe promoter-level substructure in gene regulation. morphogenesis

As described in chapter XXX, gene regulation may be thought of as a combination of cis-acting regulation by the extended promoter of a gene (including all regulatory sequences) by way of the transcription complex, and of trans-acting regulation by the transcription factor products of other genes. If we simplify the cis-action by using a phenomenological model which can be tuned to data, such as a unit or other small portion of an artificial neural network, then the full trans-acting interaction between multiple genes during development can be modelled as a larger network which can again be tuned or trained to data. The larger network will in general need to have recurrent (feedback) connections since at least some real gene regulation networks do. This is the basic modeling approach taken in [Mjolsness et al. '91], which describes how a set of recurrent neural networks can be used as a modeling language for multiple developmental processes including gene regulation within a single cell, cell-cell communication, and cell division. Such network models have been called "gene circuits", "gene regulation networks", or "genetic regulatory networks", sometimes without distinguishing the models from the actual modeled systems.

In [Mjolsness et al. '91] a number of choices were made in formulating the trainable gene regulation network models, which affect the spatial and temporal scales at which the models are likely to be useful. The dynamics was chosen to operate deterministically and continuously in time, on continuous-valued concentration-like variables, so that the dynamical equations for the network are coupled systems of ordinary differential equations (ODE's). One such form was

$$\tau_i \frac{dv_i}{dt} = g\left(\sum_j T_{ij} v_j + h_i\right) - \lambda v_i$$

in which $v_i$ is the continuous-valued state variable for gene product $i$, $T_{ij}$ is the matrix of positive or negative connections by which one transcription factor can enhance or repress another, and $g()$ is a nonlinear monotonic sigmoidal activation function.

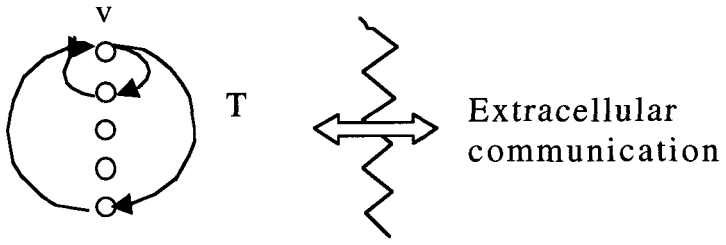


**Figure 1.** Sketch of recurrent analog neural network model for gene regulation networks. A set of analog-valued units $v$ are connected in a continuous-time, recurrent circuit by a connection matrix $T$. Communication with circuits in other cells may require additional connections, e.g. as formulated in [Mjolsness et al '91].

Such equations are often stiff due to the nonlinear transfer function $g(u)$. Optimizing the unknown parameters $T$, $\tau$, and $\lambda$ has so far proven to be computationally difficult: special versions of simulated annealing optimization [Lam and Delosme '88a, '88b] have been required for good results, e.g. to start from expression patterns derived from a known model and recover its parameters reliably [Reinitz and Sharp '95]. Informatics work on improving this situation could be important.

In addition to the analog circuit model, the framework of [Mjolsness et al. '91] also proposes a dynamic *grammar* by which multiple

biological mechanisms can be modeled by networks and then combined into a consistent overall dynamical model. The grammar/circuit combination has some similarities to hybrid models incorporating Discrete Event Systems and ODE's. In this way one can for example combine intracellular and intercellular regulation network submodels. The grammar is also suitable for implementation in object-oriented computer simulation programs.

## 2. Case Studies

In this section, some of the literature on trainable gene circuit models which have been fit to *Drosophila* gene expression patterns is reviewed.

### 2.1 Gap Gene Expression

Such gene regulation network models can be tuned or "trained" with real gene expression data, and then used to make robust and at least qualitatively correct experimental predictions, as was shown in [Reinitz et al. '92]. In that study the goal was to understand the network of gap genes expressed in bands (domains) along the anterior-posterior (A-P) axis of the very early embryo (the syncytial blastoderm) of *Drosophila*. This experimental system has the advantage that there are no cell membranes between adjacent cell nuclei, so elaborate cell-cell signalling mechanisms do not need to be modeled. Also *Drosophila* is an easy species to manipulate genetically, as for example "saturation mutagenesis" - finding all the genes affecting a particular process - is possible.

Positional information along the A-P axis of the syncytial blastoderm is encoded in a succession of different ways during development. At first the main encoding is a roughly exponential gradient of *bicoid (bcd)* protein imposed by the mother fly, along with maternal *hunchback (hb)* expression. These provide gene regulation network inputs to the gap genes: *Kruppel (Kr), knirps (kni), giant (gt), tailless (tll)*, and *hunchback (hb)* again. These each establish one or two broad domains of expression along the A-P axis. The gap genes then serve as network inputs to the pair-rule genes including *even-skipped (eve)* and *fushi tarazu (ftz)*, which establish narrow, precise stripes of expression and precise positional coding. These in turn provide input to segment-polarity genes such as *engrailed* and

*wingless* which are the first to retain their expression pattern into adulthood. For example, *engrailed* is expressed in bands just one cell wide which define the anterior borders of the parasegments. Introductions to the relevant *Drosophila* developmental biology may be found in [Lawrence 92] and [Alberts et al. 94].

The first computer experiments with fitting such analog gene regulation nets to real expression data concerned the establishment of the broad gap gene domains (excluding the extreme ends of the A-P axis) from maternally supplied initial conditions, by a gene regulation network in which all gap genes interact with all others and *bcd* provides input to, but does not receive any input from, the gap genes.

Figure 2 shows the experimentally observed and model-fitted curves for gap gene expression. They are in qualitative agreement, which is the most that can be expected from the expression data that was available at the time. The extra dip in *gt* expression could not be predicted by the model, which may be interpreted as an indication of the role of circuit components not included in the model.

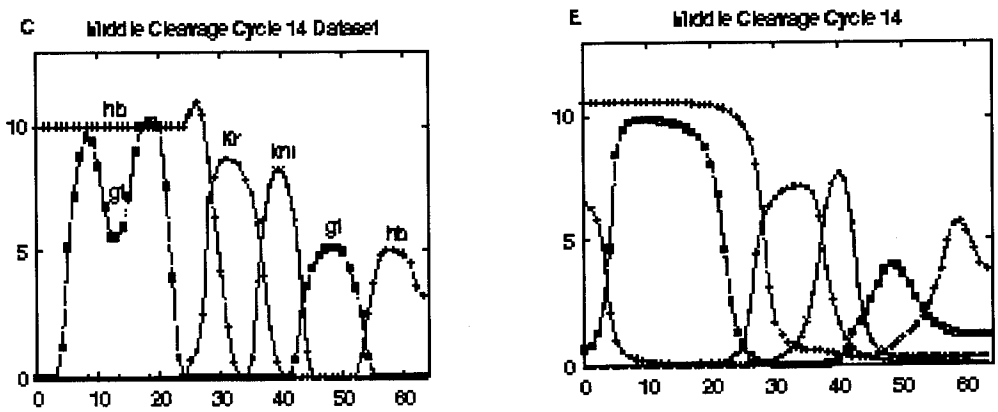


**Figure 2.** Data and model for gap gene circuit. Horizontal axes are nuclei along lateral midline from anterior to posterior. Vertical axes are relative concentrations, estimated from fluorescence tagged antibodies for the data plot (left) or output from a circuit model fit to expression data using a nonlinear least squares criterion and simulated annealing optimization (right). From [Reinitz et al. '92].

The most important predictions of the model concerned the anomalous dose-response observed by [Driever and Nusslein-Volhard '88]. Figure 3 shows the prediction in detail; it may be

summarized by saying that positional information for the gap gene system is specified cooperatively by maternal *bcd* and *hb*. This qualitative behavior was observed to be robust over many runs of the simulated annealing parameter-fitting procedure, and therefore taken to be a prediction of the model. Essential features of the cooperative control of positional information by maternal *bcd* and *hb* were verified experimentally in [Simpson-Brose et al. '94]. The gap gene model prediction and the experiment ocurred independently of one another.

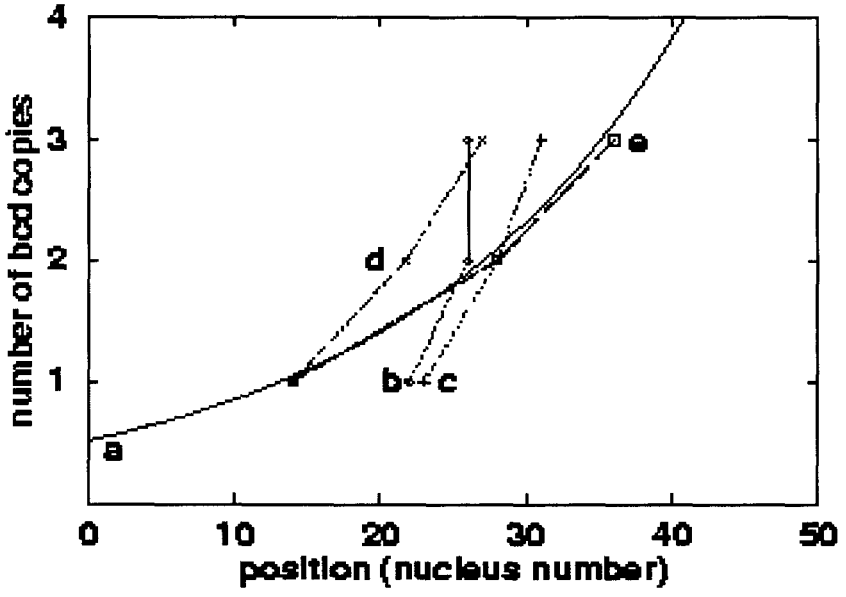


**Figure 3.** Predictions of the model as *bicoid* dosage is increased: location of selected landmarks along A-P (horizontal) axis vs. number of bcd copies (vertical axis). (a) Displacement of a landmark (anterior margin of the *Kr* domain) expected if it were determined by reading off a fixed concentration value of maternal Bicoid protein alone. (b-c) Smaller displacement of the same landmark (anterior margin of the *Kr* domain) predicted by model. (A retrodiction.) (d) Observed anomalously small displacement of a related landmark: the first *eve* stripe, not available in the gap gene model but expected to be offset anteriorly from the *Kr* landmark. Note anomalously high slope compared to a, but as in b,c. (e) Prediction: return to the behavior of (a) if maternal *hunchback* is set equal to zero. From [Reinitz et al. '92].

## 2.2 Eve Stripe Expression

Following the gap gene computer experiments, [Reinitz and Sharp '95] went on to perform a detailed study of the gap gene circuit as extended to include the first of the pair-rule genes, *eve*. The further observations which could be included in this model allowed an important milestone to be reached: not only qualitative behaviors, but also the circuit parameter signs and rough magnitudes became reproducible from one optimization run to another, and some parameters such as connections to eve were still more reproducible. Hence, far more could be predicted. For example the diffusion constant for *eve* was much lower than for other transcription factors in successful runs. This has an experimental interpretation: *eve* mRNA is expressed in the outer part of each future cell just as the cell membranes are invaginating into the blastoderm embryo, providing an apical obstruction to diffusion.

More importantly, each of the eight boundaries of the four central stripes of *eve* expression could be assigned a particular gap gene as the essential controller of that boundary. This picture is in agreement with experimental results with the possible exception of the posterior border of *eve* stripe 3, the interpretation of which is an interesting point of disagreement [Small et al. '96, Reinitz and Sharp '95, Frasch and Levine '87] and a possible focal point for further laboratory and/or computer experiments.

Further experimental understanding of the gap genes' influence on eve expression is obtained in [Reinitz et al. '98], where it is shown that the fact that *eve* is unregulated by other pair-rule genes can be understood by the phase of its periodic spatial pattern: no other phase offset pattern of pair-rule expression (e.g. the phase-shifted patterns of *hairy* or *fushi-tarazu*) can be produced from gap gene input alone.

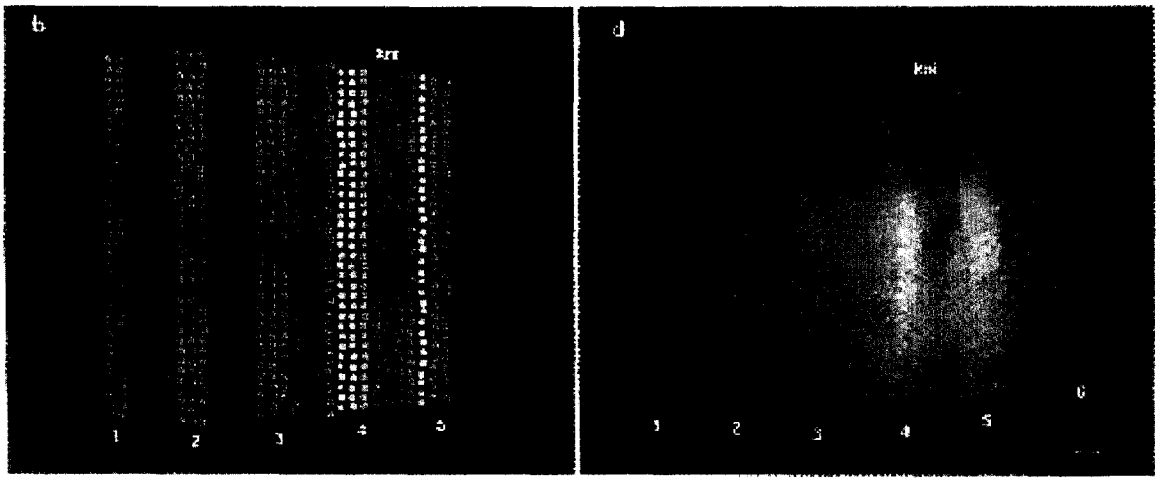

**Figure 4.** Drosophila *eve* stripe expression in model (right) and data (left). Green: *eve* expression, red: *kni* expression. From [Reinitz and Sharp '95]. Courtesy J. Reinitz and D. H. Sharp.

Related work on modeling the gap gene and *eve* system of A-P axis positional information in *Drosophila* includes [Hamahashi and Kitano '98].

## 2.3 Neurogenesis and Cell-Cell Signaling

The syncytial blastoderm is very favorable, but also very unusual, as morphogenetic systems go because there is no cell membrane interposed between nearby cell nuclei and therefore the elaborate mechanisms of cell-cell signaling do not come into play. But if we are to model development in its generality it is essential to include signaling along with gene regulation networks. As a first attempt in this direction, we have modeled the selection of particular cells in an epithelial sheet (later in *Drosophila* development) to become neuroblasts. Virtually the same gene network is thought to be involved in the selection of particular cells in wing imaginal disks to be sensor organ precursors. The essential molecule to add is the Notch receptor, a membrane-bound receptor protein responsible for receiving the intercellular signals which mediate this selection process. It binds to a ligand molecule ("Delta" for this system) on neighboring cells. Recent experiments [Schroeter et al. '98] indicate that it acts on the nucleus (following activation by a ligand on another cell) by having an intracellular domain cleaved off and transported there. Variants of the Notch receptor occur in many

developmental subsystems where a subpopulation of cells must be picked out, in *Drosophila* and homologously across many species.

In [Marnellos '97] and [Marnellos and Mjolsness '98a, '98b] are reported computer experiments incorporating both intracellular and intercellular components in a gene regulation network model of neurogenesis. A minimal gene circuit model with lateral inhibition (such as depicted in Figure 5) was not quite sufficient to produce the observed patterns of selection robustly. Incorporating a denser intracellular connection matrix and/or the dynamic effects of delamination on the geometry of cell/cell contact area produced better results. However, the "data" to which the fits were made was highly abstracted from real gene expression data so it is premature to draw a unique biological hypothesis from the model. Figure 6 shows the resulting model behavior in the case of dense interconnections.

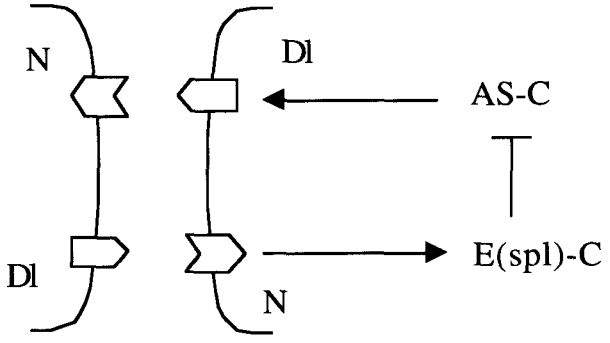


**Figure 5.** A hypothesized minimal gene regulation circuit for lateral inhibition mediated by Notch and Delta. Redrawn from [Heitzler et al '96, Figure 6]. Two neighboring cells express Notch (N) and Delta (Dl) at their surfaces. Notch positively regulates transcription of genes of the Enhancer-of-split complex E(spl)-C, which negatively regulate transcription of genes of the *achaete-scute* complex (AS-C), which positively regulate transcription of *Delta*. Curved boundaries are the cell membranes between two neighboring cells. Related circuit diagrams have been suggested elsewhere e.g. [Lewis '96].

Related work on Notch-mediated signaling in *Drosophila* developmental models includes the appearance of Notch and Delta in the ommatidia model of [Morohashi and Kitano '98].

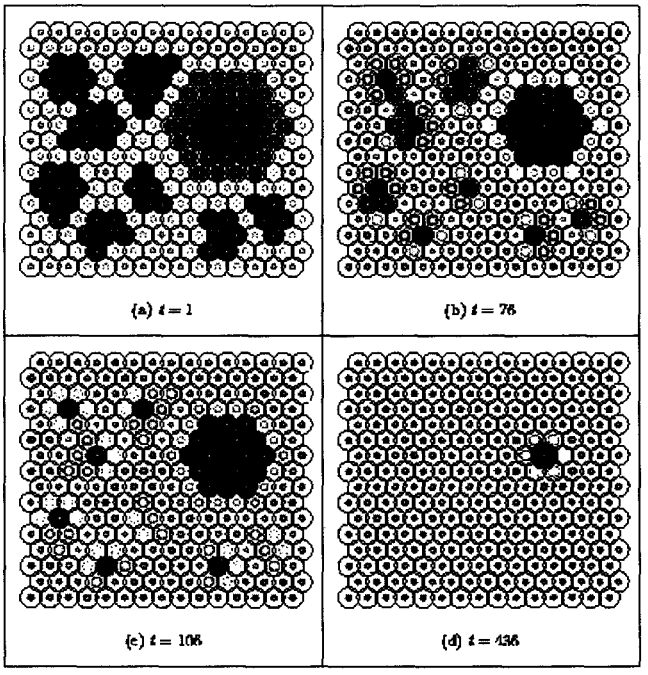

**Figure 6.** Cluster resolution. A circuit "trained" to resolve simple proneural cluster configurations into individual neuroblasts (or sensory organ precursor cells) is tested on more complex and irregular configurations. In this case each cluster was successfully resolved into a single neuroblast, but the large clusters resolve more slowly. From [Marnellos and Mjolsness '98a].

## 3 Extending the Model to Include Promoter Substructure

A very important scientific problem is to understand the influence of promoter substructure on *eve* stripe formation. The *eve* promoter has many transcription factor binding sites, some of which are grouped more or less tightly into promoter elements such as the stripe 2 "minimal stripe element" (MSE 2) [Small et al. '92], or a similar less tightly clustered element for stripes 3 and 7 [Small et al. '96]. As an example of the scientific problems that are raised, if is *hb* an enhancer for MSE 2 but an inhibitor for MSE 3, what is its net effect on *eve* and can it change sign [Reinitz et al. '98]? And how are we to understand the action of "silencer" elements such as the one apparently responsible for long-range repression of *zen* by *dorsal* [Gray et al. '95]? Such questions point to the need for at least one additional level of complication in the phenomenological models of

gene networks whose application is described above, to describe the substructure of promoters: binding sites, their interactions, and promoter elements. Otherwise the relevant experiments cannot even be described, let alone predicted, with network models.

In [Small et al. '92] an informal model for activation of MSE 2 is suggested: it is activated by *bcd* and *hb* "in concert", and repressed by *gt* anteriorly and *Kr* posteriorly. A simple "analog logic" expression for the activation of MSE 2 in terms of variables taking values in [0,1] might then be [GRN '98]:

$$u_{MSE2} = (bcd + \gamma \times hb)(1 - gt)(1 - Kr)$$
$$v_{MSE2} = g(u_{MSE2})$$

where $\gamma$ is a weight on the relative contribution of *hb* vs *bcd*. A similar simplified formula for the model of [Small et al. '96, figure 8] for MSE 3 could be for example:

$$u_{MSE3} = Dstat(1 - hb)(1 - kni)$$
$$v_{MSE3} = g(u_{MSE3})$$

(We omit direct activation of MSE3 by *tailless (tll)* since *tll* represses *kni* [Pankratz et al. '89] which represses MSE3.) The rate of *eve* transcription would be approximated by a further analog logic formula including a weighted "or" of the MSE activations $v_{MSE2}$ and $v_{MSE3}$.

The validation or invalidation of such formulae and their interpretation in terms of more detailed models will require a quantitative treatement of the relevant expression data which is not yet available. It may also lead to fitting the parameters in quantitative network models of promoter-level substructure within a gene regulation network.

### 3.1 An Example: Hierarchical Cooperative Activation

As an example of such a gene network model incorporating promoter level substructure, I introduce here a "Hierarchical Cooperative Activation" (HCA) model for the degree of activation of a transcription complex. It at least seems more descriptive of known

mechanisms than a previous attempt to derive phenomenological recurrent neural network equations as an approximation to gene regulation dynamics [Mjolsness et al. '91]. An earlier suggestion for including promoter-level substructure in gene regulation networks is described in [Sharp et al. '93]. The present HCA model is more detailed but has not been fit to any experimental data yet and is therefore quite speculative: perhaps a next stage of successful modeling will include some of the following ingredients.

The basic idea of the model is to use an equilibrium statistical mechanics model (complete with partition functions valid for dilute solutions [Hill '85]) of "cooperative activation" in activating a protein complex. Such a model can be constructed from the following partition function, which is essentially the Monod-Wyman-Changeux model for a concerted state change among subunits [Hill '85]:

$$Z = K\prod_b (1 + K_{bj} v_j) + \prod_b (1 + \hat{K}_{bj} v_j)$$

in which the probability of activation of some complex is determined by relative binding constants for each component $b$ of the complex in the active and inactive states, but there are no other interactions. As before, $v_j$ represents the concentration of gene product $j$ of a gene circuit. For this partition function, given a global active or inactive state, all binding sites are independent of one another. For example the components could be the occupants of all the binding sites $b$ within a particular regulatory region of a eukaryotic promoter. This conditional independence leads to the products over the binding sites in the expression for $Z$. There are two such products because there is an additional bit of global state which can be "active" or "inactive". Each binding site is presumably specialized to a particular transcription factor (but see below for heterodimers); the other $K$'s are set to zero.

In the special case where all the binding sites $b$ specialized to a particular transcription factor $j$ have roughly equal binding constants $K$, we can simplify $Z$:

$$Z \approx K\prod_j (1 + K_j v_j)^{m_j} + \prod_j (1 + \hat{K}_j v_j)^{m_j}$$

For this model the probability of activation of the complex under consideration is

$$P = g(u) = \frac{Ku^m}{1 + Ku^m}$$

$$u = \prod_j \left( \frac{1 + K_j v_j}{1 + \hat{K}_j v_j} \right)^{m_j / m}$$

$$u \approx 1 + \sum_j \frac{m_j}{m} \left( K_j - \hat{K}_j \right) v_j \qquad \text{(if } Kv << 1\text{)}$$

Here $m$ can be chosen as an average of $m_j$ over the relevant gene products $j$. (The final line suggests a neural-network like approximation for $u$, although in that regime $g$ could be linearized also.) But we will not restrict all consideration to this specialized case.

Given such a model of "cooperative activation", we'd like to use it hierarchically: to describe the activation of promoter "modules" or "elements" in terms of transcription factor concentrations, and then again to describe the activation of the whole transcription complex in terms of the "concentrations" of active promoter elements, which are proportional to their activities. The resulting bare-bones hierarchical model would replace the neural-net activation dynamics

$$\tau_i \frac{dv_i}{dt} = [transcribing]_i - \lambda_i v_i$$

$$[transcribing]_i = g(u_i)$$

$$u_i = \sum_j T_{ij} v_j + h_i$$

with the two-level model

$$\tau_i \frac{dv_i}{dt} = [transcribing]_i - \lambda_i v_i$$

$$[transcribing]_i = g(u_i) = \frac{Ku_i}{1 + Ku_i}$$

$$u_i = \prod_\alpha \left( \frac{1 + K_\alpha P_\alpha}{1 + \hat{K}_\alpha P_\alpha} \right)$$

and

$$P_\alpha = g(u_\alpha) = \frac{K{u_\alpha}^m}{1 + K{u_\alpha}^m}$$

$$u_\alpha = \prod_j \left( \frac{1 + K_j v_j}{1 + \hat{K}_j v_j} \right)^{m_{\alpha j}/m}$$

However, we have the opportunity to include a few more biological mechanisms at this point. One is the possibility that, as in the Endo16 model of [Yuh et al.], the hierarchy could go much deeper than two levels - especially if transcription complex formation is a sequential process. Another significant mechanism is competitive binding within a promoter element, for which the 4-term product of two 2-term binding-site partition functions is replaced with one three-term function by excluding the configuration in which both competing sites are occupied:

$$Z_{bb'} = (1 + \sum_j A_{bj} K_j v_j + \sum_k A_{b'k} K_k v_k)$$

$$\hat{Z}_{bb'} = (1 + \sum_j A_{bj} \hat{K}_j v_j + \sum_k A_{b'k} \hat{K}_k v_k)$$

(where A =0 or 1 describes which transcription factors bind to which sites) with corresponding modifications to the update equation. Also homodimeric and heterodimeric transcription factor binding are easy to accommodate with appropriate concentration products in more general one-site and two-site partition functions:

$$Z_b^{(1)} = (1 + \sum_{jk} A_{bjk} K_{jk} v_j v_k)$$

$$\hat{Z}_b^{(1)} = (1 + \sum_{jk} A_{bjk} \hat{K}_{jk} v_j v_k)$$

$$Z_{bb'}^{(2)} = (1 + \sum_{jk} A_{bjk} K_{jk} v_j v_k + \sum_{lp} A_{b'lp} K_{lp} v_l v_p)$$

$$\hat{Z}_{bb'}^{(2)} = (1 + \sum_{jk} A_{bjk} \hat{K}_{jk} v_j v_k + \sum_{lp} A_{b'lp} \hat{K}_{lp} v_l v_p)$$

Note that monomers are a special case of dimers in which the k index takes a special value for which $v_k$ is defined as a constant. Transcription factor trimers and higher order subcomplexes at adjacent binding sites could be described by suitable generalizations of these expressions.

Similarly, constitutive transcription factor binding with activation by phosphorylation or dephosphorylation can be described with minor modifications of the appropriate one-site or two-site partition functions. For example one could use Michaelis-Menton kinetics in steady-state for phosphorylation and dephosphorylation, and the one-site partition functions would become

$$Z_b^{(1)} = (1 + \sum_{jk} A_{bjk,lm} K_{jk}^{eff} v_j v_k x_l / (x_l + y_m))$$

$$\hat{Z}_b^{(1)} = (1 + \sum_{jk} A_{bjk,lm} \hat{K}_{jk}^{eff} v_j v_k x_l / (x_l + y_m))$$

where $x_{jk}$ is proportional to the concentration of a kinase for the bound j/k dimer (with proportionality constants depending on the catalytic reaction rates) and $y_{jk}$ is proportional to a corresponding phosphatase concentration. Also $A_{bjk,lm} \leq A_{bjk}$, so that the extra indices $l$ and $m$ just specify the relevant kinase(s) and phosphatase(s) from a kinase network. For example MAP kinase mediated signaling could be modeled as activating a gene regulation network by this mechanism.

Given such one-site and two-site partition functions, the overall partition function for a promoter element in terms of its binding sites is:

$$Z_\alpha = K_\alpha \left( \prod_{b|C=0} Z_b^{(1)} \right) \left( \prod_{bb'|C=1} Z_{bb'}^{(2)} \right) + \left( \prod_{b|C=0} \hat{Z}_b^{(1)} \right) \left( \prod_{bb'|C=1} \hat{Z}_{bb'}^{(2)} \right).$$

Silencers are just particular promoter elements with sufficiently strong negative regulation of transcription to veto any other elements.

The resulting model (Figure 7) can describe promoter elements, silencer regions, dimeric and competitive binding, and constitutive transcription factor binding, among other mechanisms. The price is that there are considerably more unknown parameters in the model - not exponentially many as in the general N-binding site partition function, but enough to pose a challenge to model-fitting procedures and data sets.

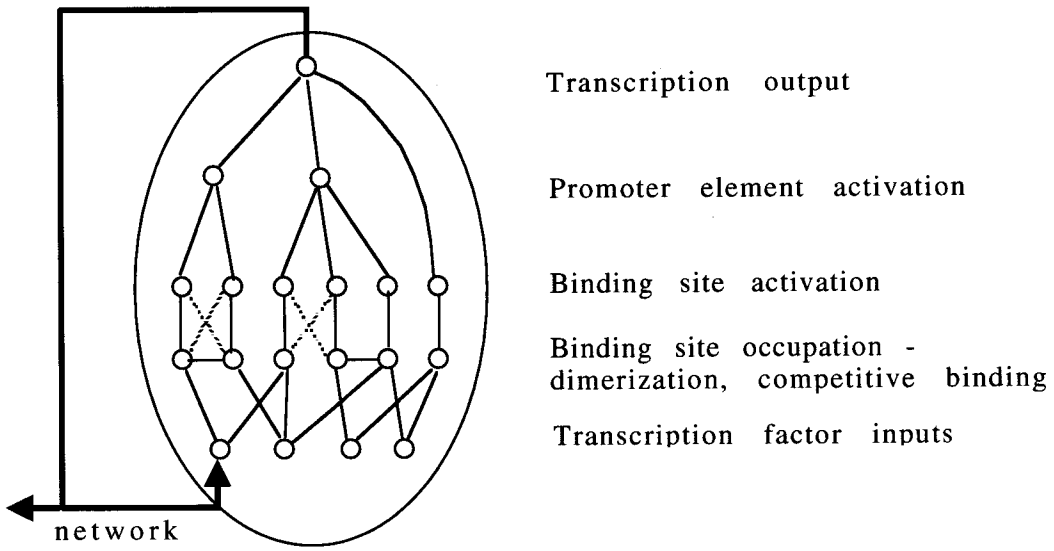


**Figure 7.** Hierarchical Cooperative Activation (HCA) model for promoter substructure within a gene "node" in a gene regulation network. Different layers of sub-nodes have different forms of dynamics. This network could be used to selectively expand some or all of the nodes in Figure 1, for example just the "*eve*" gene in a network for the gap genes and *eve*.

## 4 Conclusion

Gene regulation networks have been applied to model several episodes in the development of *Drosophila*, successfully making contact with experimental results. A variety of biological mechanisms including intercellular signaling can now be included in such models. We proposed a new version of gene regulation network models for use in describing experiments which involve promoter substructure, such as transcription factor binding sites or promoter regulatory elements.

## Acknowledgements

This work reflects ideas which arose in discussions with Hamid Bolouri, Michael Gibson, George Marnellos, John Reinitz, David Sharp, and Barbara Wold. It was supported by funding from the Office of Naval Research and the NASA Advanced Concepts program.

## References


[Alberts et al. 94] B. Alberts, D. Bray, J. Lewis, M. Raff, K. Roberts, J. D. Watson, The Molecular Biology of the Cell, 3rd edition, pp. 1077-1103, 1994.

[Driever and Nusslein-Volhard '88] W. Driever and C. Nusslein-Volhard, "A Gradient of *Bicoid* Protein in *Drosophila* Embryos". Cell 54:83-93, 1988.

[Frasch and Levine '87] M. Frasch and M. Levine, "Complementary Patterns of *even-skipped* and *fushi tarazu* Expression Involve Their Differential Regulation by a Common Set of Segmentation Genes in *Drosophila*". Genes Dev. 2:981-995, 1995.

[GRN '98]. Gene Regulation Network discussion group: H. Bolouri, M. Gibson, T. Mann, and E. Mjolsness, 1998.

[Gray et al. '95], S. Gray, H. Cai, S. Barol, and M. Levine, "Transcriptional Repression in the Drosophila Embryo". Philosophical Transactions R. Soc. Lond. B 349:257-262, 1995.

[Hamahashi and Kitano '98] S. Hamahashi and H. Kitano, "Simulation of *Drosophila* Embryogenesis", Artificial Life VI: Proceedings of the Sixth International Conference on Artificial Life, eds. C. Adami, R. Belew, H. Kitano, and C. Taylor, pp. 151-160, MIT Press 1998.

[Heitzler et al. '96] P. Heitzler, M. Bourouis, L. Ruel, C. Carteret and P. Simpson, "Genes of the *Enhancer of split* and *achaete-scute* Complexes are Required for a Regulatory Loop between *Notch* and *Delta* during Lateral Signalling in *Drosophila*", Development 122:161-171, 1996.

[Hill '85] T. L. Hill, Cooperativity Theory in Biochemistry: Steady-State and Equilibrium Systems. Springer Series in Molecular Biology, Springer-Verlag, 1985. See especially pp. 6-8, 35-36, and 79-81.

[Lam and Delosme '88a] J. Lam and J. M. Delosme, "An Efficient Simulated Annealing Schedule: Derivation". Technical Report 8816, Yale University Electrical Engineering Department, New Haven CT, 1988.

[Lam and Delosme '88b] J. Lam and J. M. Delosme, "An Efficient Simulated Annealing Schedule: Implementation and Evaluation". Technical Report 8817, Yale University Electrical Engineering Department, New Haven CT, 1988.

[Lawrence '92] P. Lawrence, The Making of a Fly. Blackwell Scientific Publications 1992.

[Lewis '96] J. Lewis, "Neurogenic Genes and Vertebrate Neurogenesis", Current Opinion in Neurobiology 6:3-10, 1996.

[Marnellos '97], G. Marnellos, "Gene Network Models Applied to Questions in Development and Evolution". Ph.D. Disseration, Yale University, 1997.

[Marnellos and Mjolsness '98a], G. Marnellos and E. Mjolsness, "A Gene Network Approach to Modeling Early Neurogenesis in Drosophila", in Pacific Symposium on Biocomputing '98, pp. 30-41, World Scientific 1998.

[Marnellos and Mjolsness '98b], G. Marnellos and E. Mjolsness, "Probing the Dynamics of Cell Differentiation in a Model of *Drosophila* Neurogenesis". Artificial Life VI: Proceedings of the Sixth International Conference on Artificial Life, eds. C. Adami, R. Belew, H. Kitano, and C. Taylor, pp. 161-170, MIT Press 1998.

[Mjolsness et al. '91] E. Mjolsness, D. H. Sharp, and J. Reinitz, "A Connectionist Model of Development". Journal of Theoretical Biology 152:429-453, 1991.

[Morohashi and Kitano '98] M. Morohashi and H. Kitano, "A Method to Reconstruct genetic Networks Applied to the Development of *Drosophila's* Eye", Artificial Life VI: Proceedings of the Sixth International Conference on Artificial Life, eds. C. Adami, R. Belew, H. Kitano, and C. Taylor, pp. 72-80, MIT Press 1998.

[Pankratz et al. '89] M. J. Pankratz, M. Hoch, E. Seifert, H. Jackle, "Kruppel Requirement for Knirps Enhancemant Reflects Overlapping Gap Gene Activities in the *Drosophila* Embryo". Nature 341:337-340, 1989.

[Reinitz et al. '92] J. Reinitz, E. Mjolsness, and D. H. Sharp, "Model for Cooperative Control of Positional Information in *Drosophila* by Bicoid and Maternal Hunchback". Journal of Experimental Zoology 271:47-56, 1995. First available (including predictions) in 1992 as Technical Report LAUR-92-2942 Los Alamos National Laboratory, and posted on the web in 1993 as URL ftp://sunsite.unc.edu/pub/academic/biology/ecology+evolution/papers/drosophila_theory/Positional_Info.ps, 1993.

[Reinitz and Sharp '95] J. Reinitz and D. H. Sharp, "Mechanism of *eve* Stripe Formation". Mechanisms of Development 49:133-158, 1995.

[Reinitz et al. '98] J. Reinitz, D. Kosman, C. E. Vanario-Alonso, and D. H. Sharp, "Stripe Forming Architecture of the Gap Gene System", Technical Report LAUR-98-1762, Los Alamos National Laboratory, 1998.

[Schroeter et al. '98] E. H. Schroeter, J. A. Kisslinger and R. Kopan, "Notch-1 Signalling Requires Ligand-Induced Proteolytic Release of Intracellular Domain". Nature 393:382-386, 1998.

[Simpson-Brose et al. '94] M. Simpson-Brose, J. Triesman, and C. Desplan, "Synergy Between Two Morphogens, Bicoid and Hunchback, is Required for Anterior Patterning in *Drosophila*". Cell 78:855-865, 1994.

[Sharp et al. '93] D. H. Sharp, J. Reinitz, and E. Mjolsness, "Multiscale Models of Developmental Processes". Open Systems and Information Dynamics 2:1,1-10, Nicholas Copernicus University Press, Torun, Poland, 1993.

[Small et al. '92] S. Small, A. Blair, and M. Levine, "Regulation *of even-skipped* Stripe 2 in the Drosophila Embryo". The EMBO Jounral 11:11, pp. 4047-4057, 1992.

[Small et al. '96] S. Small, A. Blair, and M. Levine, "Regulation of Two Pair-Rule Stripes by a Single Enhancer in the *Drosophila* Embryo". Developmental Biology 175:314-324, 1996.

[Yuh et al. '98] C.-H. Yuh, H. Bolouri, and E. H. Davidson, "Genomic Cis-Regulatory Logic: Experimental and Computational Analysis of a Sea Urchin Gene". Science 279:1896-1902, 1998.